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# ASSESSMENT OF URINARY 8-HYDROXYDEOXYGUANOSINE LEVEL IN DIABETIC CANCER PATIENTS

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**ABSTRACT:** Both diabetes mellitus and cancer are characterised by higher levels of oxidative stress and production of free radicals, which further complicate the control and outcomes of these diseases. The aim of this study was to assess the level of urinary 8-Hydroxydeoxyguanosine in diabetic cancer patients as a biomarker of cellular oxidative stress for both disease progression and chemotherapy administration. A controlled prospective observational study carried out on 100 diabetic patients newly diagnosed with diverse cancer types eligible for different chemotherapeutic protocols at the oncology unit. Urinary 8-Hydroxydeoxyguanosine level assessed at the baseline (before the required chemotherapy protocol schedule), and the 2nd reading (at the end of the required chemotherapy protocol schedule). Results showed that there was a significant ($p<0.05$) increase the urinary 8-Hydroxydeoxyguanosine level between the baseline and 2nd readings (27.04±4.33 ng/dl) vs (30.77±4.63ng/dl), and between the baseline and 2nd readings at 7-day course (25.96±4.21ng/dl) vs (28.16±5.27ng/dl), at 14-day course (27.76±5.33 ng/dl) vs (31.56±4.47ng/dl), and at 21-day course (27.22±4.16 ng/dl) vs (31.40±4.24ng/dl). In conclusion, this study presented that oxidative stress based on elevation of the urinary 8-OHdG level is related to diabetes mellitus and cancer which is further boosted during chemotherapy administration.

**INTRODUCTION:** Different solid and hematologic cancers occurred in diabetic patients. Thus diabetes mellitus (DM) and cancer associated with lower health outcomes [1]. Free radicals are reactive oxygen species (ROS) and have a tendency to donate oxygen to other substances. Therefore, they are unstable, highly reactive and can generate a second free radical, which then go on to react with a new target [2]. The major types of free radicals in living organisms include superoxide radical ($O_2^{\cdot}$), hydroxyl radical ($^{\cdot}OH$) [3].

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There is a normal balance mechanism to keep free radicals under acceptable levels by the action of endogenous antioxidants. A disturbance in this balance creates an overproduction of oxidants or underproduction of antioxidants leads to what's called oxidative stress causing damages and producing mutations that initiate oxidative events [4]. The net result is excessive production of these free radicals, which instantly attack and damage cellular macromolecules mainly proteins, lipids, carbohydrates and nucleic acids [5]. Oxidative stress linked with development of many medical conditions including, DM, cancer, cardiovascular diseases, chronic inflammation, and neuro-degenerative disorders [6].

One of the main difficulties is a direct measurement of ROS because of their short lifetime.

The best example is hydroxyl free radical, the most harmful one, has a lifespan of 0.1ns. ROS-induced modification of a purine residue in DNA leads to formation of water - soluble and renally excreted derivative called 8-hydroxydeoxyguanosine (8-OHdG) which is prevalent in cancer cells [7-9]. Urinary 8-OHdG is now considered to be an important biomarker of cellular oxidative stress in patients with DM and its level increases accordance with severity of diabetic complications [10].

**Aim of the study**: The objective of this study is to evaluate the urinary 8-Hydroxydeoxyguanosine level as a biomarker of disease progression (DM and cancer) before and after chemotherapy administration in diabetic cancer patients.

**Ethical Approval:** Approval of this study was granted from the Ethical Committee of Marmara University-Institute of Health Sciences**.**

**Methods:** This was a controlled prospective observational study carried out on 100 diabetic patients newly diagnosed with diverse types of cancers eligible for different chemotherapeutic protocols at the oncology unit of Dr. Lütfi Kırdar Kartal Teaching and Research Hospital (Istanbul-Turkey). The study participants were recruited between September 2014 and April 2015. Patients who candidate to receive a weekly dose of the chemotherapeutic regimen were followed by six cycles (a total duration of 42 days); patients who candidate to receive the chemotherapeutic regimen every 14 days were followed by three cycles (a total duration of 42 days); patients who eligible to receive the chemotherapeutic regimen every 21 days were followed by three cycles (a total duration of 63 days).

Inclusion criteria included patients over the age of 18 years, newly diagnosed cancer patients eligible for chemotherapy with a diagnosis of DM. Patients willing to participate were provided with additional written information. They were asked to sign the study consent form, and if unable to sign, their caregivers were asked to sign on their behalf. Exclusion criteria included patients with neo-adjuvant chemotherapy, patients who were receiving radiotherapy concomitantly, and patients who expressed willingness to withdraw from the study.

Urine samples for assessment of the urinary 8-OHdG level were collected from the participants at the baseline (before starting the chemotherapeutic protocol schedule), and the 2nd reading (at the end of the required chemotherapy protocol schedule). Urine samples were stored and frozen at –70°C until analysed. They were centrifuged at 2000 rpm for 10 min to remove the particulate matter and after proper dilution the supernatant was used for the determination of 8-OHdG by a competitive ELISA assay performed according to the manufacturer's instructions.

**Statistical Analyses**: The SPSS 16.0 Package was used for statistical analysis. Continuous variables were expressed as mean ± SD and categorical variables were reported as number (frequency). Continuous variables were not disturbed normally. Therefore, Wilcoxon test was performed on differences between the baselines, and 2nd readings of the required chemotherapy protocol schedules. Subgroup analysis, such as duration of chemotherapy courses (7 days, 14 days and 21 days) was tested by Kruska-Walis method as a non-parametric test of ANOVA. The results were assumed significant when the $p < 0.05$ threshold reached by all statistical analyses.

**RESULTS:** The characteristics of the study population are presented in **Table 1**. The mean age of patients is 61.82± 8.62 years. Most of the patients were females (64%), had a previous family history of cancer (61%), noncigarette smokers (85%), married (98%), and had a primary level of education (61%).

**TABLE 1: DEMOGRAPHIC PATIENTS' CHARACTERISTICS OF DIABETIC CANCER PATIENTS**

| Parameter | Patient Group n= 100 (%) |
|---|---|
| Gender Males | 36 (36%) |
| Females | 64 (64%) |
| Mean Age (year) ±SD | 61.82± 8.62 |
| Cancer Family History Yes | 61 (61%) |
| No | 39 (39%) |
| Marital State Married | 98 (98%) |
| Single | 2 (4%) |
| Cigaratte Smoking Yes | 15 (15 %) |
| No | 85 (85%) |
| Education Level Yes (Primary) | 61 (61%) |
| Yes (Secondary) | 2 (2%) |
| No | 37 (37%) |

Clinical characteristics of DM are shown in **Table 2**. The majority of patients were suffering from type 2 DM (99%). The mean duration of DM was

7.62±5.99 years with the last diabetic clinic visit of around one year. The vast majority of co-morbidities with DM were hypertension and hyperlipidaemia. Co-morbidities of DM+ hypertension constituted about (33%). On the other hand, co-morbidities of DM+hypertension+ hyperlipidemia constituted (31%).

**TABLE 2: CLINICAL DIABETES MELLITUS CHARACTERISTICS OF DIABETIC CANCER PATIENTS**

| Parameter | Patient group n= 100(%) |
|---|---|
| Type of DM Type 1 | 1 (1%) |
| Type 2 | 99 (99%) |
| DM duration (year) ±SD | 7.62±5.99 |
| Last Diabetic Clinic Visit (year) ±SD | 1.18±0.55 |
| Comorbidities DM+HT | 33 (33%) |
| DM+HT+HL | 31 (31%) |
| DM+HT+others | 9 (9%) |
| DM Medications Metformin | 37 (37%) |
| Insulin | 16 (16%) |
| Metformin+Insulin | 13 (13%) |
| Metformin+Gliclizide | 14 (14%) |
| Metformin+others | 20 (20%) |

DM=diabetes Mellitus, HT=Hypertension, HL=Hyperlipidemia

Regarding medicines used for the treatment of DM in diabetic cancer patients, there was a major usage of metformin (37%) followed by insulin (16%) and of metformin+ gliclizide (14%). Cancer characteristics are presented in **Table 3**. Breast carcinoma was the most common type of cancer (25%) followed by Non Samll Cell Lung Carcinoma (NSCLC) (14%). Most of the participants recieved chemotherapy regimens every 21 days (67%) followed by regimens every 7 days (20%), and every 14 days (13%).

**TABLE 3: CANCER AND CHEMOTHERAPY PROTOCOLS' CHARACTERISTICS OF DIABETIC CANCER PATIENTS**

| Parameter | Patient Group n= 100(%) |
|---|---|
| Cancer Type Breast CA* | 25 (25%) |
| Pancreas CA | 6 (6%) |
| NSCL CA | 14 (14%) |
| Non-Hodgkin's Lymphoma | 5 (5%) |
| Rectum CA | 9 (9%) |
| Colon CA | 5 (5%) |
| Stomach CA | 4 (4%) |
| Others | 32 (32%) |
| Previous Chemo-radiotherapy | |
| Yes | 40 (40%) |
| No | 60 (60%) |
| Cancer Therapy Schedule | |
| Every 7 days | 20 (20%) |
| Every 14 days | 13 (13%) |
| Every 21 days | 67 (67%) |

The urinary 8-OHdG level is presented in **Table 4** and showed that there was a significant increase ($p<0.05$) between the baseline and 2nd readings at the end of the required chemotherapeutic schedule (27.04±4.33 ng/dl) vs (30.77±4.63ng/dl).

The urinary 8-OHdG level based on chemotherapy protocol schedule is presented in **Table 5**. There was a significant increase ($p<0.05$) between the baseline and 2nd readings at 7-day course (25.96±4.21ng/dl) vs (28.16±5.27ng/dl), at 14-day course (27.76±5.33 ng/dl) vs (31.56±4.47ng/dl), and at 21-day course (27.22±4.16 ng/dl) vs (31.40±4.24ng/dl). There was also a significant increase ($p<0.05$) between the 2nd readings of the 7-day and 14-day course (28.16±5.27ng/dl) vs (31.56±4.47ng/dl ng/dl), and of the 7-day and the 21-day course (28.16±5.27ng/dl) vs (31.40±4.24 ng/dl).

**TABLE 4: COMPARISON OF URINARY 8-OH LEVEL BEFORE AND AFTER THE REQUIED CHEMOTHERAPY PROTOCOL SCHEDULE OF DIABETIC CANCER PATIENTS**

| Parameter | Patients n=100 (±SD) | p-value |
|---|---|---|
| Baseline 8-OHdG Reading (ng/dl) | 27.04±4.33 | 0.0001 |
| 2nd 8-OHdG Reading (ng/dl) | 30.77±4.63 * | |

* $p<0.05$ siginificance at 95% Confidence Interval within the groups

Baseline Reading= before starting the chemotherapeutic regimen,

2nd Reading= at the end of the requied chemotherapy protocol schedule

**TABLE 5: COMPARISON OF URINARY 8-OH LEVEL BETWEEN GROUPS REGARDING CHEMOTHERAPY COURSE OF DIABETIC CANCER PATIENTS**

| Chemotherapy Course | Patients | Baseline 8-OHdG Reading (±SD) (ng/dl) | 2nd 8-OHdG Reading (±SD) (ng/dl) | p-value |
|---|---|---|---|---|
| 7-day (6 cycles) | n=20 | 25.96±4.21 | 28.16±5.27 * | 0.029 |
| 14-day (3 cycles) | n=13 | 27.76±5.33 | 31.56±4.47 *a | 0.016 |
| 21-day (3 cycles) | n=67 | 27.22±4.16 | 31.40±4.24 *a | 0.0001 |

* -$p<0.05$ siginificance at 95% Confidence Interval within the groups

a =$p<0.05$ siginificance at 95% Confidence Interval when the values between groups are compared

Baseline Reading= before starting the chemotherapeutic regimen,

2nd Reading= at the end of the required chemotherapy protocol schedule.

**DISCUSSION:** Diabetes and cancer have a great influence on general patients' health. Many

epidemiologic findings indicate that diabetic patients are at significantly higher risk for cancer development. On the other hand, DM is a more common problem in patients with advanced cancer than in normal population [11].

The results of our study found that the majority of patients were suffering from type 2 DM (99%). These findings coincided with other epidemiological studies about the association of cancer and type 2 DM due to increased patients' age, elevated insulin level and insulin resistance [12].

As presented in **Table 3**, breast carcinoma was the most common type of cancer (25%). This high occurrence standed with many studies which showed that DM associated with 13- 25% higher risk of breast cancer. Those studies found that the high occurrence of breast cancer and DM may related to multiple factors, including insulin resistance, hyper insulinemia, hyper glycemia, and accompanying obesity (high level of C-peptide and insulin in pre-diabetic phase) [13]. The urinary 8-OHdG level showed a significant increase ($p<0.05$) between the baseline and 2nd readings at the end of the required chemotherapeutic schedule, as well as a significant increase ($p<0.05$) between the baseline and 2nd readings at 7-day course at 14-day course, and at 21-day course. There was also a significant increase ($p<0.05$) between the 2nd readings of the 7-day and 14-day course, and of the 7-day and the 21-day course.

Literatures reported that during chemotherapy, some of the commonly used anticancer agents such as doxorubicin, cyclophosphamide, cisplatin, vinca alkaloids, antifolates, nucleoside and nucleotide analogues can cause production of ROS [14].

In addition, elevated 8-OHdG has detected in various tumours and is proportional to the degree of histological malignancy such as ovarian cancer [15]. Higher levels of 8-OHdG were also detected in blood levels of breast cancer patients majorly those who also suffering from DM [16]. The 8-OHdG/ creatinine ratio was found to be higher in urine of patients with bladder carcinoma and colorectal cancer [17, 18].

Both types of DM are characterised by increased oxidative stress and a permanent inflammatory condition that persists for years or decades and reducing intracellular antioxidant capacity [19]. Free radicals are formed in DM through different mechanisms, including glucose oxidation, non-enzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins which promotes development DM complications [20]. It has been found that ROS accumulation disrupts transmission pathways between the insulin receptor and the glucose transport system leading to insulin resistance and then the development of diabetic complications including retinopathy and nephropathy [21].

Hence, elevated levels of urinary 8-OHdG as a biomarker of oxidative stress (ROS) are observed in patients with type 1 and 2 DM as well as in cancer [22, 23].

These data were supported by a study of Takeshi Nishikawa et al. [24] who found that hyperglycemia increase urinary 8-OHdG level in patients with type 2 DM. Our results were also corresponded with literature data of Lily L. Wu et al. [25], and Soo Shin et al. [26] who reported elevation of the urinary 8-OHdG level in diabetic patients with hyperglycemia, and the level of urinary 8-OHdG in diabetes correlated with severity of diabetic nephropathy and retinopathy.

**CONCLUSION:** The results of this study found that many medical conditions including, DM and cancer were associated with increased oxidative stress based on elevation of the urinary 8-OHdG level and their progression further augmented during chemotherapy administration.

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